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EFFECTS OF PREVIOUS NITROGEN ADDITION ON CHLORINE IN FOREST SOIL, SOIL SOLUTION AND BIOMASS

Abstract

There is increasing evidence that forests and forest soil contribute to the signature of chlorine composition in water bodies. However, little is known about the potential effects of land management activities on chlorine biogeochemistry. This study examines the effects of previous nitrogen addition on chlorine chemistry in a *Pinus sylvestris* (L.) forest located in south-central Sweden (60°00' N, 13°43' E). Repeated addition of nitrogen to study plots over a 20-year period resulted in total additions of 0, 450 and 900 kg N ha⁻¹. Soil samples were collected before harvesting, and soil solution and biomass were sampled following final felling.

Contrary to previous findings, we found no clear evidence that previous addition of nitrogen had hampered the formation of organic chlorine in the organic soil layer. We suggest that the effects of nitrogen addition on chlorination processes are not seen in the surface soil, but are instead manifested in the migration of organic matter in the mineral soil. Soil organic matter from the E-horizon had a lower chlorination degree in the nitrogen-amended plots than in the control plots. In addition, we observed lower Cl⁻ levels in the seedling needles following high nitrogen fertilization (900N) than in 450 N or control. These results add on the importance of studying chlorine dynamics with focus on what chlorinated soil organic matter can be resistant to degradation compared to average soil organic matter and what can be leached as a result of harvesting and available for vegetation.

Keywords

Chlorine, chloride, soil, soil solution, nitrogen, forest

Introduction

Inorganic chlorine (chloride; Cl^-) has been seen as an inexpensive and suitable tracer of soil and ground water solution movements (Herczeg and Leaney 2011; Hruška et al. 2012) and studies using Cl^- as a water tracer has been a foundation for contaminant transport models (e.g. Kirchner et al. 2000). However, several studies have indicated that Cl^- can be immobilized in ecosystems by different processes, including: i) ion exchange (Viers et al. 2011), ii) adsorption onto iron and aluminium oxides (Nodvin et al. 1986), iii) uptake by vegetation (Ohrui et al. 1996; White et al. 2001; Lovett et al. 2005), iv) uptake by the microbial community (Bastviken et al. 2007), v) conversion to organic forms of chlorine (e.g. Myneni 2002; Öberg 2003; Bastviken et al. 2007), and especially high chlorination rates are found in coniferous forest soils (Gustavsson et al. 2012). In addition, Cl^- can be released by rock weathering from some rare types of bedrock, constituting extra input in addition to atmospheric deposition (Lovett et al. 2005). These processes may result in a net retention of Cl^- in ecosystems on varying time scales when internal chlorine pools are increasing in magnitude or a net release of Cl^- when internal pools are declining. Hence, it appears that soil sometimes acts as a sink for Cl^- and sometimes as a source.

The conditions under which soil acts as a Cl^- sink, or as a source, are not clear (Bastviken et al. 2006), although there are indications that several environmental factors may have an effect. One is the water residence time. The longer the water residence time, the longer the time for interactions with the soil, i.e. the more Cl_{org}

will be formed (Bastviken et al. 2006). It has also been reported that the addition of nitrogen at amounts corresponding to forest fertilization seems to hamper the formation of organic chlorine (Cl_{org}) (Öberg et al. 1996; Johansson et al. 2001). In addition, forest harvesting experiments have shown that clear-cutting generally leads to increased leaching of Cl^- (Kauffman et al. 2003; Lovett et al. 2005; Mannerkoski et al. 2005). Several factors related to forestry such as fertilization and harvesting is likely to have an effect on Cl^- cycling as they are changing the ecosystem to a great deal, but on what level and to what extent is unknown. Since Cl^- is frequently used as a tracer to determine soil solution flows, increased knowledge about chlorine transport will improve our understanding of how solutes other than chlorine move through the soil. This is of importance in fields such as hydrology, biogeochemistry, terrestrial ecology and soil remediation.

The current study investigates the influence of nitrogen on levels of inorganic and organic chlorine in soil in a *Pinus sylvestris* (L.) forest after repeated nitrogen addition followed by an investigation of levels of chlorine in soil solution and biomass after final felling. We hypothesize that i) the amount of chlorinated organic matter in soil will decrease with increasing fertilization intensity due to a decrease of the organic layer Cl pool, ii) the leachable chlorine following forest harvesting decreases with increasing N fertilization intensity, which also will affect the biomass pool. The increase is negatively related to the Cl:C ratio in the organic layer.

Materials and methods

The study was conducted in an experimental forest, 165 Hagfors, in south-central Sweden (60°00' N, 13°43' E), hereafter referred to as Hagfors, consisting of a *P.*

sylvestris stand, in which the experiment was established in 1981. Further details on the Hagfors experiment can be found in Ring et al. (2011, 2013). The annual mean temperature in the area is 3.5 °C and the annual mean precipitation is 671 mm (Alexandersson et al. 2001). The upper part of the sandy-silty till was podzolized. The median deposition of Cl^- was 2-18 kg ha⁻¹ (1997-2010) at a station with open collectors approximately 50 km southwest from the experimental forest (operated by Swedish Environmental Research Institute; <http://www.krondroppsnatet.ivl.se/>).

The original purpose of the experiment at Hagfors was to determine stemwood growth at different fertilization intensities. Seven treatments were allocated to 30×30 m² study plots in a randomized block design with three replicates. In the present study, three of these treatments were investigated, namely 0, 450 and 900 kg N ha⁻¹ (total N dose applied, denoted 0N, 450N and 900N). In the 450N treatment, 150 kg N ha⁻¹ was applied at 8 year-intervals, i.e. in 1981, 1989 and 1997. In the 900N treatment, 150 kg N ha⁻¹ was applied at 4-year intervals, i.e. in 1981, 1985, 1989, 1993, 1997 and 2001. Nitrogen was applied as NH_4NO_3 and 1 kg B ha⁻¹ was added at each application. From 1991 onwards, dolomite was also included in the fertilizer (Ring et al. 2011).

In the spring of 2006, the *P. sylvestris* stand was harvested leaving no logging residues on the plots. In late May 2006 the plots were planted with 18-month-old *P. sylvestris* containerized seedlings at 2 m spacing.

Soil

Soil samples were collected before harvesting, in June of 2005. Samples were collected from the FH layer and from 0 to 20 cm of the mineral soil (E and Bs horizon, as described by Ring et al. (2011)). From each plot; 20 sub-samples were

collected from the FH-layer (mean soil depth for each plot varied from 34 to 48 mm) and 40 sub-samples from the mineral soil (mean soil depth for each plot varied from 64 to 86 mm for the E-horizon and from 51 to 113 mm for the samples collected from the B horizon) with a soil auger. The mineral soil samples were separated to form composite samples for each E and B horizon and plot. In the present study, sub-samples taken from the samples used in the study by Ring et al. (2011) were further prepared for chlorine analysis. Sampling was performed on each of the three replicate forest plots giving 27 samples in total.

The water content of the soil was determined by drying the soil to a constant weight at 105 °C. The soil was sieved using a 2-mm mesh and then milled to 0.5 mm. The milled soil was stored in glass bottles at room temperature until loss-on-ignition, total chlorine (TX), and total organic halogens (TOX) were analysed (see below). The samples were thoroughly mixed before the analyses were carried out. Loss on-ignition was determined by combusting the milled soil for 2 h at 620 °C, the amount of organic carbon being estimated to be 50% of the weight loss. One replicate was analysed per soil sample.

Soil solution

Soil solution was collected from the mineral soil at a depth of about 40 to 45 cm, i.e. from the lower B or upper C horizon, using ceramic suction cups (type P80, CeramTec AG, Germany). Three cups per plot were installed in the forest before harvesting in May 2005. Each cup was connected to a 1-litre glass bottle through silicone tubing. A suction pressure of 75 cbar was used for sampling, generated by manual pumping. These samples were collected about eight (in the 450N regime) or four (in the 900N regime) years after the last N application. In the present study, sub-

samples of the soil solution studied by Ring et al. (2013) were analysed regarding Cl_{org} concentration, from six occasions in the 450N regime and 13 occasions (1-3 occasions during spring and autumn during 2006-2008) in both the 0N and 900N regimes. One additional sampling was undertaken in late August 2011. Results on soil solution chemistry for Cl^- and other solutes before and after harvesting have been presented by Ring et al. (2013).

The concentration of Cl_{org} in soil solution samples was determined as adsorbable organic halogens (AOX), (EU 1996). The AOX method measures the sum of chlorine, bromine and iodine and does not distinguish between the different halogens. Since chlorine is by far the most abundant of these halogens in the soil environment (Brady et al. 2002), the mass estimates are based on the assumption that chlorine dominates in the samples. If significant amounts of other halides are present, the method will overestimate the amount of Cl_{org} in the samples. Briefly, 20-40 ml (the exact volume noted) of the soil solution sample was diluted with MilliQ water to a final volume of 100 ml in a 300-ml Erlenmeyer flask. Activated carbon (50 mg; Euroglas), acidified nitrate solution (20 ml; 0.22 M KNO_3 , 0.02 M HNO_3), and approximately 5 drops of concentrated HNO_3 (yielding pH <2) were added to the flasks and the suspension was placed on a rotary shaker (180 rpm) for 1 h. The suspension was filtered through a 0.45- μm polycarbonate filter (Euroglas), and then rinsed with an acidic nitrate solution (approximately 20 ml; 0.01 M KNO_3 , 0.001 M HNO_3) followed by acidified reverse osmosis water (approximately 20 ml, adjusted to pH 2 with HNO_3). The filter was combusted in a stream of oxygen at 1000 °C in an AOX analyser (ECS3000; Euroglas) in which the hydrogen halides formed were determined by means of microcoulometric titration with silver ions. One replicate of each sample was

analysed. The levels detected were clearly above the Cl_{org} detection limit of 1 g l^{-1} .

Blanks consisting of 100 ml of Milli-Q water, containing $<3 \text{ } \mu\text{g l}^{-1} \text{ Cl}_{\text{org}}$ were analysed as controls. Further information and chemical data on Cl^- , total organic carbon (TOC) and other solutes in soil solution can be found in Ring et al. (2013).

Vegetation on the clearcut

In late June 2011, five years after planting, needle samples were collected from ten randomly selected *P. sylvestris* seedlings per plot for nutrient analysis. At the same time, three randomly selected *P. sylvestris* seedlings per plot were harvested for measurements of above-ground biomass. A sample of the stem (a 10-mm-thick disc), a representative branch (according to size and number of needles) and a shoot produced during the previous year were taken from each seedling. The ground vegetation was sampled at the same time as seedling sampling. Ten replicates (area 30 cm x 30 cm) were taken at random from each plot and pooled to give one sample per plot. The seedling and ground vegetation material were dried at $70 \text{ }^\circ\text{C}$ to a constant weight and homogenised by grinding before analysis.

Cl_{org} and Cl^- concentrations in soil and biomass

The principle for analysing Cl_{org} and Cl^- in soil is based on the AOX analysis described above, but without the addition of activated carbon. TOX in a solid sample is analysed after washing the sample with a nitrate solution to remove inorganic halides. The washing procedure is excluded when analysing the total amount of halogens (TX), and the amount of Cl^- is obtained by subtracting the amount of organic halogens from the total amount of halogens, since this procedure has been shown to be more reliable than extracting the soil and analysing the extract by potentiometric titration (Johansson et al. 2001). Normally, chlorine is by far the most abundant

halogen in environmental samples; hence, the total mass of halogens is, by tradition, calculated as the amount of chlorine, as was done in the present study.

The concentration of Cl_{org} in solid samples was analysed according to Asplund et al. (1994). Briefly, approximately 20-30 mg of the milled sample (the exact amount was noted) was added to an acidic nitrate solution (20 ml; 0.2 M KNO₃, 0.02 M HNO₃) and shaken on a rotary shaker (180 rpm) for at least one hour. The suspension was filtered through a 0.45-µm polycarbonate filter, and the analysis then followed the AOX analysis procedure described below. One replicate of each soil and biomass sample was analysed. Blanks were analysed using the same procedure but without the addition of soil. The levels detected in all samples were clearly above the detection limit, which was approximately 1 µg Cl_{org} g⁻¹ dry weight (d.w.).

TX was determined by adding 20 mg of sieved, milled soil (0.12 mm) to a small crucible followed by direct combustion in the AOX analyser. One replicate of each soil sample was analysed. Blanks were analysed by combustion of the crucibles without the addition of soil. The levels detected in all samples were clearly above the detection limit, which was approximately 1 µg Cl_{org} g⁻¹.

Statistics

The soil chemistry and biomass data were statistically analysed using the MIXED procedure in SAS ver. 9.3 (<http://support.sas.com>). The following mixed linear model was applied:

$$y_{ij} = \mu + a_i + b_j + e_{ij}$$

where y_{ij} is the variable of interest, μ is the overall mean, a_i is the fixed effect of the N fertilization regime i , b_j is the fixed effect of block j , and e_{ij} is the residual.

Adjustment for multiple comparisons was made according to the Tukey–Kramer method. Soil solution chemistry data were analysed using a mixed linear model for repeated measurements, as described by Ring et al. (2013). Statistically significant effects were defined as $p < 0.05$.

Results

Soil

The dominating form of chlorine in the soil was Cl_{org} , about 70%. The concentration of Cl_{org} ranged from 21 to 285 $\mu\text{g Cl}_{\text{org}} \text{ g}^{-1}$ soil d.w. (dry weight) and the concentration of organic carbon ranged from 12 to 440 $\text{mg C}_{\text{org}} \text{ g}^{-1}$ soil d.w.

The amount of Cl_{org} in the surface soil layer was about eight times that in the mineral soil layer (Table 1). No differences in Cl_{org} concentration were found in the surface soil layer subjected to the nitrogen treatments. However, the concentration of Cl_{org} in the two deeper soil horizons for 900N was lower than for 450N and 0N.

In order to evaluate the fraction of chlorinated organic matter the chlorine-to-carbon (Cl:C) ratio of soil organic matter was calculated by dividing the concentration of Cl_{org} with concentration of total carbon in soil. It can be seen from the results in Table 1 that the chlorinated organic matter in the soil increases with increasing depth. The Cl:C ratio in soil from plots treated with 450 kg N ha^{-1} was about 4 times higher in the E-horizon than in the surface layer, and increased further in the Bs horizon (Figure 1). The amount of chlorinated organic matter in the soil following the 900N treatment

also increased with soil depth, but to a lesser extent than with the 450N treatment. The Cl:C ratios in the surface layer were similar for the different N additions. For the control plots with no N addition (0N), the Cl:C ratio was similar in the E and Bs horizon.

Table 1. Concentration of Cl_{org} and chlorine-to-carbon (Cl:C) ratios in different soil layers, based on measurements on three plots per treatment. Bold p -values indicate that the N fertilization regime had a significant effect. (Significant differences between different N treatments for each soil layer are indicated by different letters).

Soil layer	$Cl_{org} \mu g g^{-1} \text{ soil d.w.}$ (n=3) Median (min-max)				$Cl:C \text{ ratio}$ (n=3) Median (min-max)			
	p	0N	450N	900N	P	0N	450N	900N
FH	0.91	237 (164-285)	247 (217-282)	236 (223-259)	0.98	0.60 (0.37-0.65)	0.55 (0.49-0.64)	0.53 (0.52-0.60)
E	0.47	31 (26-40)	28 (21-32)	26 (23-29)	0.15	2.43 (2.14-3.30)	2.28 (1.65-2.44)	1.48 (1.36-1.70)
Bs	0.019	55 ^a (47-65)	59 ^a (53-66)	38 ^b (37-39)	<0.001	2.40 ^a (2.14-2.98)	3.20 ^b (2.93-3.66)	2.27 ^a (2.19-2.31)

Figure 1. Box plot showing Cl:C ratios (Cl_{org} divided by total carbon) in different soil horizons following different amounts of N fertilization.

INSERT FIGURE 1.

Soil solution

It can be seen from Figure 2 that the concentration of Cl_{org} decreased with time after harvesting. The observed concentrations after suction cup installation directly after harvesting were very high, reaching almost $1000 \mu g l^{-1}$. However, these results should be treated with caution as there are some indications that measure of total organic carbon might have been affected by the installation of lysimeters (Ring et al. 2013).

We therefore assume that this might also be the case for Cl_{org} .

Figure 2. Organic chlorine (Cl_{org}) in soil solution after harvesting ($n=3$). The dotted line indicates time for harvesting.

INSERT FIGURE 2

The concentration of Cl_{org} without fertilization (0N) was around $200 \mu\text{g l}^{-1}$ already in October 2006, six months after harvesting. The mean concentration stayed fairly constant from November 2006 to September 2007 (varying between approximately 130 and $214 \mu\text{g l}^{-1}$). From October 2007, the concentration fell to approximately $80 \mu\text{g l}^{-1}$, and dropped even more during the spring of 2008, $\sim 40 \mu\text{g l}^{-1}$. The concentration remained low, $\sim 40 \mu\text{g l}^{-1}$, in samples collected in August 2011, five years after harvesting.

Similar patterns were seen for the other treatments, but with some differences (Figure 2). For instance, the concentration of Cl_{org} in plots treated with 900N was similar to that in control plots during the autumn of 2006, but during the following spring in 2007 (one year after harvesting) the soil solution concentration of Cl_{org} in these fertilized forest plots was half the concentration found in control plots. Soil solution from 450N showed relative high Cl_{org} concentrations during the first months after harvesting. In 2008, the concentrations in the 450N plots were similar to concentrations in the 0N and 900N plots. No statistically significant differences could be seen for the different N treatments over the whole sampling period ($p = 0.17$).

The Cl:C ratios in the soil solution decreased with time after harvesting in all forest plots (Figure 3). The ratios ranged from 35 to $160 \text{ mg Cl g C}^{-1}$ during 2006 and 2007, and decreased to below $40 \text{ mg Cl g C}^{-1}$ during 2008. During spring 2007, the Cl:C ratios were lower in N-fertilized plots (900N) than in the control plots, while in 2008

and thereafter the ratios were similar. No statistically significant differences were seen between the different N treatments for the whole period ($p = 0.70$).

Fig. 3. Median chlorine-to-carbon ratio (mg Cl g C^{-1}) in soil solution after harvesting ($n= 3$).

INSERT FIGURE 3

Vegetation biomass

The concentrations of Cl^- ranged from 37 to 372 $\mu\text{g g}^{-1}$ d.w. (median: 97 $\mu\text{g g}^{-1}$ d.w.) for seedling biomass and ground vegetation (Table 2). Needles and ground vegetation had the highest Cl^- concentrations (11-372 $\mu\text{g g}^{-1}$ d.w.). The lowest Cl^- concentrations were found in stems (2-4 times lower than in needles), while the values in branches were slightly higher.

All parts of the biomass investigated had a low content of Cl_{org} relative to Cl^- , where Cl_{org} varied from 1 to 48 $\mu\text{g g}^{-1}$ d.w. (median: 6 $\mu\text{g g}^{-1}$ d.w.). Contrary to Cl^- , similar Cl_{org} concentrations were found in the investigated biomass. The percentage Cl_{org} of total chlorine in biomass averaged 9%.

Effects of N regime

The Cl^- concentrations in needles were lower in the plots with the most intensive fertilization regime (900N) (Table 2), and the effect of N regime was significant ($p=0.01$). The Cl:C ratio ranged from <0.01 to 0.94 mg g^{-1} among stem, needles and branches. We found no significant effects of N regime on the Cl^- concentrations or Cl:C ratios in stems, branches or ground vegetation. In addition, we found no correlations between either Cl^- to N or C content or Cl_{org} to the same.

Table 2. Concentrations of organic chlorine (Cl_{org}) and Cl^- in stems, branches and needles from *P. sylvestris* seedlings sampled five growing seasons following harvesting. Ground vegetation was sampled at the same time as the seedlings.

Biomass	$\text{Cl}^- \mu\text{g g}^{-1} \text{ d.w.}$ (n=3) <i>Median (min-max)</i>				$\text{Cl}_{\text{org}} \mu\text{g g}^{-1} \text{ d.w.}$ (n=3) <i>Median (min-max)</i>			
	<i>p</i>	0N	450N	900N	<i>P</i>	0N	450N	900N
Stem	0.15	64.6 (45.0-93.5)	80.1 (66.6-97.4)	54.0 (37.2-60.3)	0.84	12.4 (6.1-28.4)	6.0 (3.1-48.2)	11.5 (8.5-11.7)
Branches	0.92	78.9 (63.0-188.8)	102.4 (57.7-179.4)	107.3 (88.1-107.6)	0.63	4.9 (3.1-23.2)	4.5 (4.1-12.8)	3.0 (2.4-7.9)
Needles	0.013	214.6 ^a (201.9-227.2)	233.3 ^a (203.3-241.0)	132.6 ^b (118.2-139.9)	0.58	3.9 (3.4-6.1)	3.6 (1.2-5.2)	3.9 (2.7-19.6)
Ground vegetation	0.31	134.3 (111.9-162.9)	203.6 (185.0-372.9)	135.4 (121.8-257.7)	0.73	14.9 (8.7-29.5)	18.9 (12.2-23.4)	14.7 (13.2-16.7)

Discussion

No observable effects of N amendment on chlorine content in top-soil

Not surprisingly, adding nitrogen to forest soil can significantly affect the biogeochemical cycling of, for instance, soil organic matter (e.g. Hyvönen et al. 2008). This is of concern for chlorine cycling as it appears that the chlorination of organic matter is related to both amount of soil organic matter and the type of vegetation (Gustavsson et al. 2012). Most of the studies on chlorine biogeochemistry have been performed on the organic-rich surface soil layer. Our hypothesis was that the Cl_{org} concentration would decrease with intensified nitrogen fertilization as has been the observed in previous studies (Johansson et al. 2001, Öberg et al. 1996). In

the present study, we did not observe any effects on the long-term of nitrogen amendment on chlorine content in the organic top-soil.

One of the previous studies on the effect of nitrogen on chlorine content in soil showed decreasing concentrations of Cl_{org} in soil with increasing N amendment (Johansson et al. 2001). The lab-incubated soil was sampled from the organic-rich surface soil layer, and the concentration of Cl_{org} and the Cl:C ratio in soil were similar to those found in the present study. Johansson et al. (2001) suggest that the decrease in Cl_{org} concentration is a direct microbial response to N amendment, a hampered microbial chlorination of organic matter. The results from the laboratory incubation study probably reflect a short-term response relatively to the current study because the measuring was done within less than two months, compared to the current study that was done under field conditions and on another time scale (years *vs* months).

Opposite to the surface soil, we observed differences in chlorine content in the lower soil layers for control plots and the N amended plots. In the E horizon, the most intensive N fertilization regime (900N) led to a lower concentration of Cl_{org} than in the control plots (0N). In the Bs horizon, amendment with 450 kg N led to higher Cl:C ratios than in control plots and in plots amended with 900 kg N, which showed similar ratios to those in the control plots. As the sampling in the current study was done more than five years after the last addition of nitrogen, one explanation could be that a chlorination processes that was taken place in the surface soil would, after this time, be seen in the deeper soil horizons.

In all soil samples, we observed higher Cl:C ratio in the deeper soil layers than in the surface soil layer. Previous studies have emphasized that the migration of soil organic

matter through different soil layers is important for internal Cl cycling in soils (e.g. Öberg and Sandén 2005). Migration of chlorinated organic matter in soils is relatively new finding and few researchers have tried to determine the Cl_{org} content in different types of soil organic matter. Bastviken et al. (2007 and 2009) found that 1-10% of the Cl_{org} in coniferous soil was associated with the water-leachable fraction of organic matter. Beside formation of Cl_{org} in soil, Leri and Myneni (2010) suggest that plant litter Cl_{org} contributes to both the water soluble fraction and recalcitrant organic matter. Very little is known about the type of chlorinated organic material that is leached down the soil profile, accumulated and what time it takes.

Chlorinated organic matter in soil solution

The observed soil solution concentrations in the forest plots studied here were generally lower than $200 \mu\text{g l}^{-1}$, and only in a few cases below $40 \mu\text{g l}^{-1}$. To the best of our knowledge, only one previous field study has been performed on Cl_{org} concentrations in soil solution. Öberg and Grön (1998) analysed the soil solution at a soil depth of 45-50 cm, similar to our study, and found median concentrations of $185 \mu\text{g l}^{-1}$ ($70\text{--}250 \mu\text{g l}^{-1}$), which is similar to the current study. Higher Cl_{org} concentrations, a median of $400 \mu\text{g l}^{-1}$, ($250\text{--}1000 \mu\text{g l}^{-1}$) were observed in a laboratory soil lysimeter study by Rodstedth et al. (2003). However, the values in the laboratory study were obtained by leaching a 15 cm soil core, i.e. the surface soil, which contains more Cl_{org} than deeper soil layers. The most surprising results of the present study are the relatively high Cl:C ratios in soil solution, which varied from 35 to $160 \text{ mg Cl g C}^{-1}$ in soil solution. These ratios are much higher than the Cl:C ratios in soil core leachates ($3.1\text{--}8.5 \text{ mg Cl g C}^{-1}$) observed by Rodhstedt et al. (2003). In addition, the Cl:C ratios in bulk soil of the present study, irrespective soil horizon or

degree of N fertilization, were only 0.4 to 3.7 mg Cl g C⁻¹ in soil) which indicate a fraction of mobile chlorinated organic matter in soil. However this comparison should be treated with caution as the soil bulk sampling was done before harvest and the soil solution sampling after harvesting. Öberg and Sandén (2005) also found higher Cl:C ratios in soil leachate from 15-cm-deep soil cores than in bulk soil. Despite that, Cl:C ratios in soil leachate were general less than 10 mg Cl g C⁻¹ in the study by Öberg and Sandén (2005), which is much less than the current study. The knowledge on chlorination and transport and migration of chlorinated organic material through the soil is small, which make the interpretation of N effect difficult. If looking over the whole observation period, we cannot see a clear pattern of the influence of fertilization when we compare soil solution from the different N treatments, i.e. a decreased leaching of chlorine with increasing fertilization intensity. The chlorine soil dynamics are related to various factors besides the fertilization. One major factor is hydrology. For instance, the washout of organic matter is usually greater during spring and snow melt. The more mobile the soil organic matter, the more organic matter will leach down the soil layers. In combination with present chlorination hypotheses; chlorination of organic matter could make the organic matter more water leachable and/or a preference for chlorination of easy degradable organic matter (Leri and Myneni 2010, Öberg and Sandén 2005). During the first spring after harvesting, in 2007, we observed lower Cl:C ratios in soil solution from nitrogen-fertilized plots (however not significant for the whole period). One may hypothesize that the lower Cl:C ratios in soil solutions in N amended plots are due to lower chlorination rates in fertilized soil, i.e. N fertilization has hampered the chlorination of organic matter leading to less chlorinated organic matter in deeper soil layers. These observation

needs to be further investigated by looking into e.g. how chlorine dynamics (changes in surface soil and migration of chlorinated organic matter) are affected by N amendment in soil profiles.

Cl in biomass

The Cl^- content of plant biomass varies between plant species (Lobert et al 1999). A general requirement of $1 \text{ mg Cl}^- \text{ g}^{-1} \text{ d.w.}$ has been suggested, but Cl deficiency has been observed at $0.1\text{--}5.7 \text{ mg Cl}^- \text{ g}^{-1} \text{ d.w.}$, and toxic levels between 4 and $50 \text{ mg g}^{-1} \text{ d.w.}$ have been reported (White et al. 2001). The observed lower Cl^- levels in the seedling needles following high nitrogen fertilization (900N) than in 450 N or control, could indicate Cl^- stress. Previous nitrogen addition had no effects on seedling growth and vegetation biomass in the present study, which might support a hampered growth in N amended plots, despite more available nutrients (Johansson et al. forthcoming). In addition, the large range in the observed levels of Cl^- means that extrapolation across species and location are highly unreliable. The plant Cl_{org} content has previously been estimated to be $0.01\text{--}0.1 \text{ mg g}^{-1} \text{ d.w.}$ (Öberg et al. 2005), but this is based on scattered measurements from beech leaves, spruce needles, sphagnum moss and bulk samples of grass, and the variability between species and plant parts is not known. The concentrations of Cl_{org} in the current study are in the lower range of previous studies, with a maximal concentration of $0.04 \text{ mg g}^{-1} \text{ d.w.}$

There is little knowledge on what may influence the pool of available Cl^- to vegetation and also the Cl cycling dynamics in soil and vegetation. The reasons for the high Cl:C ratios and decrease of Cl^- in soils will remain puzzling and challenge conventional knowledge. Ring et al. (2013) observed a decrease of Cl^- in soil solution

throughout the study period. The observed Cl^- concentrations decreased over time, and at the end of the study was about 20% of the concentration measured soon after harvesting. The final Cl^- concentration measured, five years after harvesting was about 0.9 mg L^{-1} , which is similar to rain concentrations. Cl^- accounted for about 80% of the total amount of chlorine in soil solution directly after harvesting, and five years after harvesting increased to approximately 95%. Thereby the available Cl^- decreased dramatically over time, which may have had implications for the vegetation. The annual root uptake of Cl^- by Scots Pine (*Pinus sylvestris*) was found to be 9-fold larger than Cl^- demand by the tree and the excess Cl^- was returned primarily as Cl^- in throughfall and to some extent by litterfall (van den Hoof and Thiry 2012). There are however no differences in Cl^- concentrations in soil solution in the different N treatment plots, which could explain the lower Cl^- levels in seedling needles for 900N treatment.

No chloride pulse?

The export of Cl^- from soil via groundwater to surface water has been considered to be primarily controlled by Cl^- input through precipitation and, therefore, it has been assumed in many studies to be largely inert in ecosystems, with little uptake or release by vegetation or soils (Schlesinger 1997). Previous forest harvesting experiments have shown that clear-cutting generally leads to an increase in the leaching of Cl^- (Likens et al. 1970; Kauffman et al. 2003; Lovett et al. 2005). These observations strongly indicate that there is a Cl^- source in soil, and the Cl^- does not act as a conservative element.

A disturbance such as harvesting is likely to lead to increased mineralization of organic matter, which may lead to an increased release of Cl^- previously bound to the

organic matter, producing a peak in Cl^- concentration in soil solution after harvesting. In this forest experiment, the Cl^- concentration in soil solution decreased steadily after harvesting for all treatment plots (data not shown). Forest management has considerable impact on the forest ecosystem, in particular the hydrology and vegetation composition. However, due to the lack of data before harvesting we cannot elucidate if there was a chloride pulse. The chlorine dynamics in forest systems are most likely influenced by e.g. forest harvesting, which causes increased organic matter decomposition rates, but also changes vegetation composition, and reduces dry deposition (throughfall) and litterfall due to canopy removal. During the field study period, we observed a decrease in Cl_{org} concentrations in soil solution with time after harvesting. It is likely to believe that reduced evapotranspiration caused by tree removal can result in dilution of soil organic matter, which was concluded by the observation of simultaneously decrease in $\text{Cl}:\text{C}$ ratio. However, the evapotranspiration after harvest was probably not progressively reduced over the observation years and can therefore not explain the decreasing trend over several years.

Conclusions

Contrary to previous findings, fertilization with nitrogen amendment had no observable effect on chlorine content in the top-soil. We suggest that the effects of nitrogen addition on chlorine content in soil are caused by chlorination processes that are not sustained in the surface soil, but rather seen in the migration of organic matter in the mineral soil. This is in line with previous studies on chlorine biogeochemistry, in which it was emphasized that the migration of soil organic matter across different depth zones is important for internal Cl cycling in soils. The soil organic matter in the E-horizon showed a lower $\text{Cl}:\text{C}$ ratio in the nitrogen-amended plots than in the control

plots, and the organic matter in soil solution also showed lower ratios in nitrogen-amended plots during the first spring season after harvesting. These results underline the importance of studying soil chlorine dynamics in order to determine which fraction is resistant to degradation and thus selectively preserved, and which fraction is leached in relation to nitrogen fertilization and final felling.

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